

CASE REPORT

A late-onset and mild form of Charcot-Marie-Tooth disease type 2 caused by a novel splice-site mutation within the Mitofusin-2 gene

KATARZYNA KOTRUCHOW, DAGMARA KABZIŃSKA, IRENA HAUSMANOWA-PETRUSEWICZ
AND ANDRZEJ KOCHAŃSKI

Neuromuscular Unit, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Charcot-Marie-Tooth type 2A disease (CMT2A) caused by mutations in the Mitofusin 2 gene (*Mfn2*) has been shown to be an early-onset axonal neuropathy with severe clinical course in the majority of the patients. In this study we present a unique phenotype of CMT2A disease characterized by late-onset polyneuropathy with a very mild clinical course. This rare form of CMT2A disease is caused by a new splice-site (c.311+1G>T) mutation within the *MFN2* gene. Due to disturbance of the *MFN2* splicing process, this mutation generates a short transcript which encodes a very short fragment of MFN2 protein. The c.311+1G>T mutation within the *MFN2* gene results in the late-onset CMT2 disease.

Key words: Charcot-Marie-Tooth disease, late-onset polyneuropathy, Mitofusin 2, splice-site mutation

Introduction

Molecular diagnostics and genetic counseling in the late-onset Charcot-Marie-Tooth disease (CMT) are made problematic by difficulties encountered in differentiating between acquired and inherited polyneuropathy in the sporadic patients over 50. Even if a new sequence variant in one of the CMT genes is found, there is still a probability that it represents harmless polymorphism, rather than a pathogenic mutation. Mutations in the Mitofusin-2 gene have been shown to be causative for CMT2A disease (OMIM #609260) (1). In a classic paper from Chung and colleagues, mutations in the *MFN2* gene (OMIM

*608507) have been shown to segregate with two distinct phenotypes, i.e. an early-onset severe form of CMT2 disease and a late-onset one (> 10 years) characterized as a mild form of axonal neuropathy. Interestingly, in the cohort of patients reported by Chung and colleagues, only one patient began to manifest with CMT at age 50 (2). Recently Feely and co-workers characterized a group of 27 CMT2-affected patients, among which a majority were of early age (range 1-6 years) at onset, while only 4 patients started to manifest with CMT2 over the age of 10. The authors concluded that CMT2A disease is an early-onset polyneuropathy with a rather severe clinical course (only 4 out of 15 adult patients could ambulate independently by age 20) (3). Among 60 studied mutations of the *MFN2* gene, only 4 have been shown to act by way of splicing disturbance (4-7).

The vast majority of mutations within the Mitofusin-2 (*MFN2*) gene segregate with an early-onset axonal form of Charcot-Marie-Tooth disease of severe clinical course (CMT2A). In some CMT2A-affected patients even demyelination of the central nervous system is reported.

Among the pathogenic mutations of the *MFN2* gene, only 4 have been shown to disturb the splicing process. However, in the present study, we report on a new *MFN2* splice-site mutation (c.311+1G>T) segregating with a late-onset (6th decade of life) CMT2A disease with a slowly progressive and mild clinical course.

Clinical features

The family under study originates in south-western Poland. Its pedigree shows what is most probably autosomal dominant inheritance. The mother of the proband died aged 77, and was most probably CMT-affected, having gait disturbances. A son of the proband, a 37-year-old male, manifested only with *pes cavus* deformity and mild wasting of the distal leg muscles.

Now aged 61, the proband had started to complain of an unsteady gait and foot dropping from age 50, though she had never had good notings at sport at school. Diagnosis of CMT was established when she was aged 59. She showed normal mentation. Neurological examination of the patient at age 61 showed: symmetrical muscle atrophy of the small hand muscles, *pes cavus* deformity, and marked atrophy of distal muscles, observed in the lower extremities. Ankle jerks were absent, while cranial nerves were normal (Fig. 1).

In the motor fibers of the median nerve, nerve conduction velocity (NCV) was within the normal range, while compound motor action potential amplitude (CMAP) was slightly reduced (4.0 mV). CMAP was markedly reduced (to 1.0mV) in the peroneal and tibial nerves, while motor NCV in the peroneal and tibial nerves was also slightly reduced (to 33.0 m/s and 35 m/s), respectively. Sensory nerve conduction velocity (SNCV) in the median nerves was within the normal range, while sensory nerve action potential amplitude (SNAP) was reduced (4 microV). SNCV and SNAP in the sural nerve were both reduced (to 31.0 m/s; 2.0 micro V). In the muscle tibial anterior a chronic neurogenic pattern in EMG was observed. The results of standard laboratory tests (glucose, serum CK, CSF) were within the norm.

In summary, our proband presented with a late-onset, motor-sensory, primary axonal polyneuropathy expressed in the lower limbs at the electrophysiological level through a secondary demyelinating component, with a slowly progressing and mild clinical course.

Material and methods

Molecular genetic studies were performed once the informed consent of the patient had been obtained. This study was approved by the local Ethical Committee. DNA and total RNA were isolated from white blood cells of the proband. Duplication/deletion of the *PMP22* gene was first excluded using the real-time quantitative polymerase chain reaction (RQ-PCR) with TaqMan probes (8). The reference values for the dosage of the *PMP22* gene in our laboratory range from 0.700 to 1.090 in healthy individuals (9). The value for the proband was 0.989. The mitofusin-2 gene-coding sequence was divided into 16 fragments, amplified by PCR with prim-



Figure 1. The proband at the age of 61 years. There is *pes cavus* deformity and mild muscle atrophy in the distal part of the lower limbs.

ers designed by V.H. Lawson and co-workers (10), sequenced directly using a Big-Dye Terminator Sequencing Ready Reaction kit (from Applied Biosystems) and analyzed on an ABI PRISM 373 device. The *MPZ* gene coding sequence containing intron-exon boundaries was analyzed. Reverse transcription was performed with a RevertAid First Strand Synthesis Kit (Fermentas), with oligo(d)T primers, in line with the manufacturer's protocol. The cDNA was amplified using primers located at the junction of exons two and three (forward primer) and in exon six (reverse primer) and also sequenced directly using a Big-Dye Terminator Sequencing Ready Reaction kit (from Applied Biosystems) and analyzed on an ABI PRISM 373 device. Sequencing data were analyzed by comparing with a reference mRNA isoform 1 sequence NM_014874.3 and genomic sequence NG_007945.1 of the *MFN2* gene. The Protein reference sequence was NP_055689.1. DNA samples obtained from 100 healthy controls (200 chromosomes) were screened for mutation c.311+1G>T in the *MFN2* gene using the RFLP-PCR approach with *Mse I* restriction enzyme. The c.311+1G>T mutation results in the creation of a new restriction site producing two fragments (210 bp and 69bp long) instead of one PCR product (279 bp) present in the wild sequence (data not shown).

Results

Direct sequencing of the *MFN2* gene revealed a novel mutation within the fourth intron (c.311+1G>T). In the RFLP approach, this sequence variant was not observed in 100 healthy controls (200 chromosomes, 50 women and 50 men of ages ranging from 20 to 50 years). The analysis of the *MFN2* cDNA fragment of the proband re-

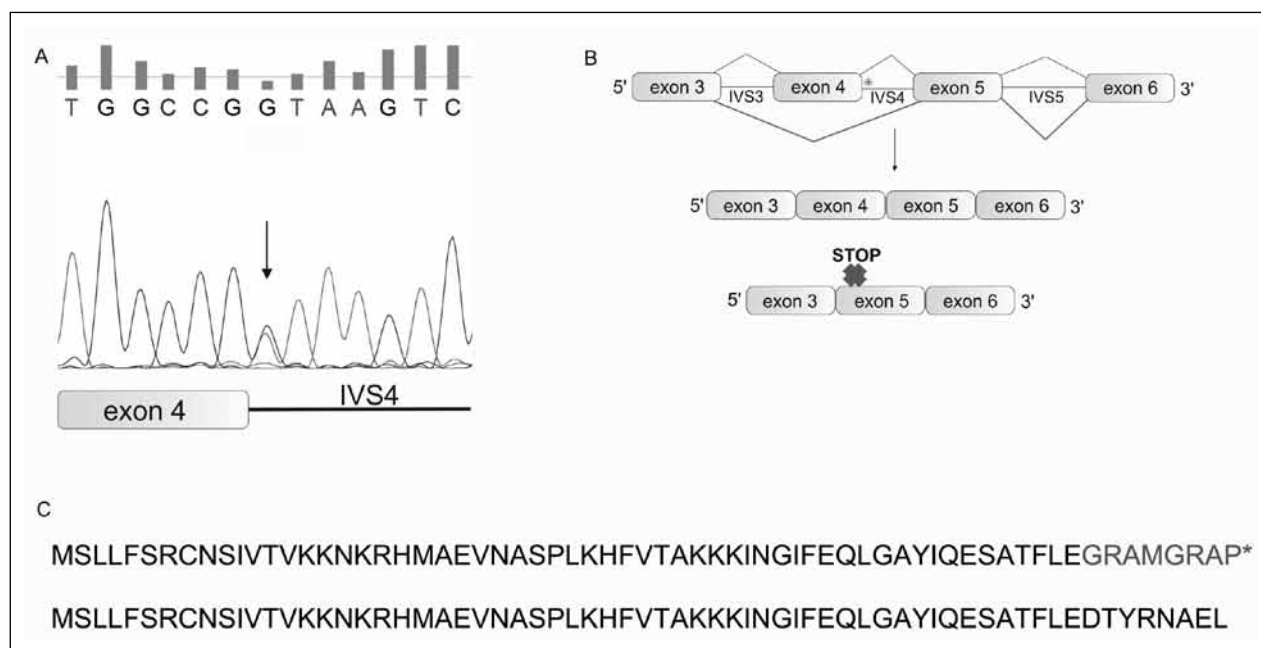


Figure 2. Chromatogram presenting heterozygous mutation in the *MFN2* gene in the genomic DNA of the proband.

Diagram presenting the location of the mutation in intron 4 (above), the correctly spliced mRNA (in the middle) and the aberrant splicing process of the *MFN2* gene with premature codon STOP indicated using a red cross (bottom). The latter results in the production of a truncated protein with only 66 amino acids, whereas the wild-type protein has 757.

Comparison of the amino-acid sequence of the short protein due to the c.311+1G>T mutation with the fragment of the native protein's amino-acid sequence.

vealed the presence of an additional band, which is from the transcript with skipped exon 4. The direct sequencing of cDNA revealed that the mutation c.311+1G>T results in the skipping of exon 4 of the *MFN2* gene (data not shown) and thus produces a short protein with a premature STOP codon (Fig. 2).

Discussion

The unusual phenotype of CMT2A disease reported in our study may be explained by reference to the mechanism of c.311+1G>T mutation. We have shown here that this mutation generates a short 394 bp transcript that corresponds by conceptual translation with a short (66-amino acid) Mfn2 protein. In functional terms, the c.311+1G>T mutation seems to act in a mild negative-dominant mechanism.

The other splice-site mutation within the *MFN2* gene (c.1392+2T>C) was shown to result in a fatal encephalopathy in 4 members of an Italian family. Interestingly, the same mutation was detected in the healthy 64-year-old female, who had had 6 spontaneous first-trimester miscarriages of unexplained etiology. The c. 1392+2T>C mutation generates 4 splicing products, which most probably act via a dominant-negative mechanism (5). Park

and colleagues have recently reported a c. 474+4A>G splice-site mutation within the *MFN2* gene which results in a rather mild phenotype of CMT2 disease with age at onset ranging from 12 to 42 years. The patients were ambulant at the moment of examination. At the electrophysiological level, CMAP was only reduced in the lower extremities. This mutation disrupted correct splicing of *MFN2* (7). The results of our study and those of other authors show that splice-site mutations within the *MFN2* gene can result in a mild form of CMT2 disease.

The c.475-1G>T mutation detected in the two patients with CMT2A disease causes deletion of 4-amino acids (p.T159-Q162del) and also segregates with a mild phenotype in two individuals, with a very mild electrophysiological expression (6).

Our study supports the thesis that splice-site, frameshift and nonsense mutations of the *MFN2* gene may be causative for a late-onset mild form of CMT2 disease. In contrast to these, missense mutations located in biologically-important domains of the *MFN2* gene are causative for early-onset and clinically-severe CMT2 disease.

The majority of *MFN2* gene mutations segregate with a phenotype of an early-onset, severe Charcot-Marie-Tooth disease of type 2 (3). Only a few patients manifesting with late-onset (over 50 years old) axonal

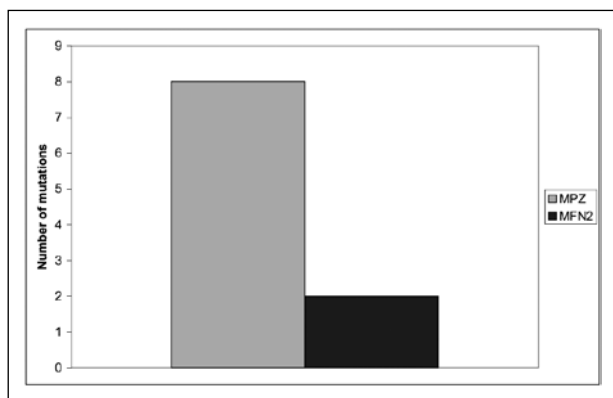


Figure 3. Presentation of the numbers of mutations that cause late-onset CMT2 disease. In the *MPZ* gene there are 8 such mutations, compared with just 2 in the *MFN2* gene. The *GJB1* gene was excluded from this summary due to its specific X-dependent mode of inheritance. The term "late-onset" refers here to the disease beginning in the 6th decade of life.

polyneuropathy have been reported to carry mutations in the *MFN2* gene (Fig. 4). To the best of our knowledge the two *MFN2* gene mutations that characterize late onset are at the end of the coding sequence, resulting in the Ala738Val and Leu753fs mutations (12, 13). The native *MFN2* protein has 757 amino acids. It is probable that the localization of the mutation could be the cause of the observed age of onset. In these cases the almost-complete protein produced may play its physiological role.

To date late-onset (6th decade of life) CMT2 disease has been shown to segregate with heterozygous mutations in the Myelin Protein Zero gene (Fig. 3). However, our study supports the idea that patients showing late-onset CMT2 disease should also be screened for mutations in the Mitofusin-2 gene. Thus, CMT2 patients negative for the *MPZ* gene should also be tested for *MFN2* gene mutations.

Acknowledgements

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